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# Six new species of *Sporothrix* from hardwood trees in Poland

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## Abstract

*Sporothrix* (*Sordariales*, *Ascomycota*) is a well-supported monophyletic lineage within the *Ophiostomatales*, species of which occur in a diverse range of habitats including on forest trees, in the soil, associated with bark beetles and mites as well as on the fruiting bodies of some *Basidiomycota*. Several species have also been reported as important human and animal pathogens. During surveys of insect- and wound-associated *Ophiostomatales* from hardwood trees in Poland, many isolates with affinity to *Sporothrix* were recovered. In the present study, six undescribed *Sporothrix* spp. collected during these surveys are characterized based on their morphological characteristics and multi-locus phylogenenetic inference. They are described as *Sporothrix cavum*, *Sporothrix cracoviensis*, *S. cryptarchum*, *S. fraxini*, *S. resoviensis*, and *S. undulata*. Two of the *Sporothrix* spp. reside in the *S. gossypina*-complex, while one forms part of the *S. stenoceras*-complex. One *Sporothrix* sp. is a member of lineage F, and two other species grouped outside any of the currently defined species complexes. All the newly described species were recovered from hardwood habitats in association with sub-cortical insects, wounds or woodpecker cavities. These species were morphologically similar, with predominantly asexual states having hyaline or lightly pigmented conidia, which produce holoblastically on denticulate conidiogenous cells. Five of the new taxa produce ascomata with necks terminating in long ostiolar hyphae and allantoid ascospores without sheaths. The results suggest that *Sporothrix* species are common members of the *Ophiostomatales* in hardwood ecosystems of Poland.

## Keywords

6 new species, bark beetle-associated fungi, *Ophiostomatales*, phylogeny, tree wounds

## Introduction

*Sporothrix* was established by Hektoen and Perkins (1900) based on the morphological description of the human pathogen, *Sporothrix schenckii*. Species of *Sporothrix* (*Ascomycota*, *Ophiostomatales*, *Ophiostomataceae*) were first accommodated in *Sporotrichum* (De Beurmann and Gougerot 1911). Until the latter half of the 20th century, these fungi were also treated in various other genera, including *Cephalosporium*, *Cladosporium* (Hedgcock 1906; Münch 1907; Lagerberg et al. 1927; Melin and Nannfeldt 1934; Siemaszko 1939; Davidson 1942; Bakshi 1950; Mathiesen-Käärik 1953; Hunt 1956), *Cylindrocephalum*, *Hormodendron* (Robak 1932), *Hyalodendron* (Goidànich 1935; Georgescu et al. 1948), and *Rhinotrichum* (Georgescu et al. 1948; Sczerbin-Parfenenko 1953), in order to accommodate the asexual morphs of *Ophiostoma*. de Hoog (1974) published a monograph of the *Sporothrix* species and proposed the placement of *S. schenckii* as the asexual morph of *O. stenoceras*. That monograph expanded the concept of *Sporothrix* and included new *Sporothrix* species causing human infections as well as those associated with wood and bark beetles.

de Hoog et al. (1985) recognized that *Sporothrix* is not a homogenous group. As DNA sequencing technology was applied to resolve taxonomic relationships for fungi, evidence emerged that *S. schenckii* is phylogenetically related to species of *Ophiostoma* (Berbee and Taylor 1992; Hausner et al. 1993, 2000). In these studies, species producing only sporothrix-like asexual states were treated as members of the *S. schenckii*–*O. stenoceras* complex in *Ophiostoma sensu lato* (De Beer et al. 2003; Villarreal et al. 2005; Roets et al. 2006; Zipfel et al. 2006; De Meyer et al. 2008; Linnakoski et al. 2010; Kamgan Nkuekam et al. 2012). The genus *Sporothrix* was recently redefined and emended based on the analysis of partial 18S and 28S rDNA sequences for species in the *Ophiostomatales* (De Beer et al. 2016). *Sporothrix* was consequently separated from species of *Ophiostoma* and various complexes were defined within *Sporothrix*. *Sporothrix* is now defined as one of nine relatively clearly defined genera in the *Ophiostomataceae* (De Beer and Wingfield 2013; De Beer et al. 2013a, 2013b, 2016).

As currently recognized, *Sporothrix* includes 56 species (De Beer et al. 2016; Ngubane et al. 2018; Wang et al. 2019; Musvuugwa et al. 2020), which are characterized by their dark brown to black, globose ascomata with elongated necks up to 1600 µm, occasionally terminating in an ostiole, often surrounded by ostiolar hyphae. Ascospores are usually curved and lunate to reniform, without a sheath (De Beer and Wingfield 2013). The asexual states have conidiophores that proliferate sympodially and produce hyaline or occasionally pigmented conidia on denticulate conidiogenous cells (De Beer and Wingfield 2013).

*Sporothrix* includes a large assemblage of species that are widely distributed across various climatic zones of the world (De Beer and Wingfield 2013; De Beer et al. 2016). Species also occupy a wide range of habitats. The greatest numbers of species are found on bark, in the infructescences of *Protea* spp. and on the wood of different forest trees (e.g., Roets et al. 2008, 2009, 2013; De Errasti et al. 2016). Other species have been described from soil, bark beetles, ambrosia beetles, mites, and from the fruiting bodies

of basidiomycetes (e.g., Constantinescu and Ryman 1989; Marmolejo and Butin 1990; De Meyer et al. 2008; Roets et al. 2008; De Errasti et al. 2016). Several species are also well-known as human and animal pathogens (Travassos and Lloyd 1980; Summerbell et al. 1993; Barros et al. 2004; Lòpez-Romero et al. 2011; Zhang et al. 2015).

Jankowiak et al. (2019a) conducted the first extensive survey of fungal associates of hardwood-infesting bark and ambrosia beetles in Poland. In the same year, *Ophiostomatales* associated with wounds on hardwood trees were also studied in Poland (Jankowiak et al. 2019b). These studies reported several *Sporothrix* species, which were apparently new to science, but names were not provided for them. In addition, one unknown *Sporothrix* species was isolated from cavities of woodpeckers in Poland (Jankowiak et al. 2019c). In this study, morphological characters and DNA sequence data for the ITS region (ITS1–5.8S–ITS2) and three protein coding genes (β-tubulin, calmodulin, translation elongation factor 1-α) were analyzed to characterize six new species of *Sporothrix*. These were compared with closely related known species and formal descriptions have been provided for them.

## Materials and methods

### Fungal isolates

The collection details for the isolates included in the present study (Table 1) are provided in previous studies (Jankowiak et al. 2019a, 2019b, 2019c). The cultures are maintained in the culture collection of the Department of Forest Ecosystems Protection, University of Agriculture in Krakow, Poland, and in the culture collection of the Natural Resources Institute Finland (Luke), Helsinki, Finland. The ex-type isolates and representative isolates of the new species described were deposited in the culture collection (**CBS**) of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands. Dried cultures were deposited as holotype specimens in the Mycological Herbarium (**O**), Natural History Museum, University of Oslo, Norway.

### Microscopy and growth studies

Morphological characters were examined for selected isolates as well as for the herbarium specimens selected as types. Cultures were grown on 2% Malt Extrat Agar (**MEA**) made up of 20 g Bacto malt extract, 20 g agar Bacto agar powder (Becton Dickinson and Company, Franklin Lakes, USA) in 1 l deionized water. In attempts to induce the formation of ascomata, autoclaved twigs of host trees including the bark were placed at the centres of agar plates containing MEA. Fungal cultures were derived from single spores. To promote the production of ascomata, single conidial isolates were crossed in all possible combinations, following the technique described by Grobbelaar et al. (2009). These cultures were incubated at 25 °C and monitored regularly for the appearance of fruiting structures.

**Table 1.** Isolates used in the present study.

| Fungal species | Previous identification[A] | Isolate no. | | | Source | Site | GenBank accessions[E] | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | CBS[B] | O-F[C] | KFL=NRFI[D] | | | ITS1-5.8S-ITS2 | βT | TEF 1-α | CAL |
| *Sporothrix cracoviensis* sp. nov | *Sporothrix* sp. 7 | CBS 147940 | | KFL17FRJTD | Adult of *Trypodendron domesticum* on *Fagus sylvatica* | Krzeszowice | MH283148 | MH283365 | MH283500 | MH283526 |
| | | CBS 147939 | | KFL2114bRJTD | Adult of *Trypodendron domesticum* on *Fagus sylvatica* | Krzeszowice | MH283149 | MH283366 | MH283501 | MH283527 |
| | | CBS 147941 | O-F-258629 | KFL2514aRJTD[F] | Adult of *Trypodendron domesticum* on *Fagus sylvatica* | Krzeszowice | **MW768963** | MH283367 | MH283502 | MH283528 |
| | | CBS 147942[ET] | O-F-258628 | KFL2514bRJTD | Adult of *Trypodendron domesticum* on *Fagus sylvatica* | Krzeszowice | **MW768964** | MH283368 | MH283503 | MH283529 |
| *Sporothrix fraxini* sp. nov | *Sporothrix* sp. 8 | CBS 147936[ET] | O-F-258630 | KFL21BS16bRJHV | Gallery of *Hylesinus varius* on *Fraxinus excelsior* | Zbylitowska Góra | MH283150 | MH283370 | MH283504 | MH283530 |
| | | CBS 147938[F] | O-F-258631 | KFL21BS16dRJHV | Gallery of *Hylesinus varius* on *Fraxinus excelsior* | Zbylitowska Góra | **MW768968** | MH283371 | **MW768973** | MH283531 |
| | | CBS 147937 | | KFL21BS16cRJHV | Gallery of *Hylesinus varius* on *Fraxinus excelsior* | Zbylitowska Góra | MH283151 | MH283372 | MH283505 | MH283532 |
| *Sporothrix resoviensis* sp. nov | *Sporothrix* sp. 10 | CBS 147927[ET] | O-F-258632 | KFL204ABRZN16AO | Wound on *Betula pendula* | Borownica | MH740962 | MH741100 | MH741189 | MH741228 |
| *Sporothrix cryptarchum* sp. nov. | *Sporothrix* sp. 11 | | | KFL1097NOL16RJ | Wound on *Alnus incana* | Wierzchosławice | MH740963 | MH741101 | MH741190 | MH741229 |
| | | | | KFL1146NDB16RJ | Wound on *Quercus robur* | Ispina | MH740964 | MH741102 | MH741191 | MH741230 |
| | | CBS 147935 | | KFL48716NDBRJ | Wound on *Quercus robur* | Wierzchosławice | **MW768967** | MH741103 | MH741192 | **MW768977** |
| | | CBS 147934[ET] | O-F-258633 | KFL410DB16bRJCU | Adult of *Cryptarcha undata* | Wierzchosławice | **MW768966** | MH741104 | MH741193 | MH741231 |
| | | CBS 147933[E] | O-F-258634 | KFL404DB16aRJCU | Adult of *Cryptarcha undata* | Wierzchosławice | **MW768965** | MH741105 | MH741194 | MH741232 |
| *Sporothrix undulata* sp. nov. | *Sporothrix* sp. 12 | CBS 147931[E] | O-F-258636 | KFL13NDB15bRJ | Wound on *Quercus robur* | Wierzchosławice | MH740965 | MH741106 | **MW768974** | **MW768978** |
| | | CBS 147930 | | KFL12NDBCZ15RJ | Wound on *Quercus rubra* | Wierzchosławice | MH740967 | MH741108 | MH741196 | **MW768979** |
| | | CBS 147928 | | KFL221NBK16RJ | Wound on *Fagus sylvatica* | Czajowice | MH740970 | MH741112 | MH741199 | MH741235 |
| | | CBS 147932 | | KFL430NDB16RJ | Wound on *Quercus robur* | Ispina | MH740971 | MH741113 | MH741200 | MH741236 |
| | | | | KFL1099NOLCZ16RJ | Wound on *Alnus incana* | Wierzchosławice | MH740973 | MH741115 | MH741202 | MH741237 |
| | | | | KFL1140NDB16bRJ | Wound on *Quercus robur* | Ispina | MH740975 | MH741117 | MH741203 | MH741238 |
| | | | | KFL6117NWB17RJ | Wound on *Salix fragilis* | Babimost | **MW768970** | MH741119 | MH741204 | **MW768980** |
| | | CBS 147929[ET] | O-F-258635 | KFL398DB16RJEG | Adult of *Epuraea guttata* | Wierzchosławice | MH740976 | MH741121 | MH741205 | MH741239 |
| | | | | KFL404DB16bRJCU | Adult of *Cryptarcha undata* | Wierzchosławice | **MW768969** | MH741124 | MH741208 | MH741242 |
| *Sporothrix cavum* sp. nov | *Sporothrix* sp. 18 | CBS 147943[ET] | O-F-258637 | KFL42215aDRJ | Cavity of *Dendrocopos major* on *Salix fragilis* | Kraków | MF782813 | MF782850 | **MW768972** | **MW768976** |
| | | | O-F-258638 | KFL35614DRJ[F] | Cavity of *Dendrocopos medius* on *Malus domestica* | Książ Wielki | MF782814 | MF782851 | **MW768971** | **MW768975** |

[A] Isolates collected and identified during previous surveys in Poland (Jankowiak et al. 2019a, 2019b, 2019c). *Sporothrix* sp. 18 in the study of Jankowiak et al. (2019c) was labelled as *Sporothrix* sp.

[B] CBS Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands.

[C] Herbarium of the Natural History Museum, University of Oslo, Norway,

[D] KFL Culture collection of the Department of Forest Ecosystems Protection, University of Agriculture in Krakow, Poland; NRIF The Natural Resources Institute Finland (Luke), Helsinki, Finland.

[E] ITS1-5.8S-ITS2-ITS2 = the internal transcribed spacer 1 and 2 regions of the nuclear ribosomal DNA gene, 5.8S rRNA gene; βT= Beta-tubulin; TEF1-α = Translation elongation factor 1-alpha; CAL = Calmodulin.

[F] Isolates used in growth and morphological studies; [T]type strain

Sequences obtained during the survey in this study are indicated in bold.

Morphological features were examined by mounting fungal tissue in 80% lactic acid on glass slides, and fruiting structures were observed using a Nikon Eclipse 50*i* microscope (Nikon Corporation, Tokyo, Japan) with an Invenio 5S digital camera (DeltaPix, Maalov, Denmark) to capture photographic images. Microscopy followed the technique described by Kamgan Nkuekam et al. (2011). Colour designations were based on the colour charts of Kornerup and Wanscher (1978).

For each taxonomically relevant structure, fifty measurements were made, when possible, using the Coolview 1.6.0 software (Precoptic, Warsaw, Poland). Averages, ranges and standard deviations were calculated for the measurements, and these are presented in the format '(min–)(mean–SD)–(mean+SD)(–max)'.

Growth characteristics for the novel species were determined by analysing the radial growth for 12 isolates (two for each species) (Table 1). Agar disks (5 mm diam.) were cut from the actively growing margins of fungal colonies and these disks were placed at the centres of plates containing 2% MEA. Four replicate plates for each of the six putative new species were incubated at temperatures between 5, and 35 °C at 5 °C intervals. The radial growth (two measurements perpendicular to each other per plate) was determined 14 d after inoculation, and growth rates were calculated as mm/d.

## PCR, sequencing and phylogenetic analyses

DNA extractions were performed as described by Jankowiak et al. (2019d). For sequencing and phylogenetic analyses, four loci were amplified: the internal transcribed spacer region (ITS, consisting of ITS1, 5.8S, and ITS2), beta tubulin (βT), calmodulin (CAL), and the translation elongation factor 1-alpha (TEF1-α). The primers used for PCR and sequencing of the various gene regions were as follows: ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) for ITS; T10 (O'Donnell and Cigelnik 1997) or Bt2a together with Bt2b (Glass and Donaldson 1995) for βT; F-728F (Carbone and Kohn 1999) and EF2 (O'Donnell et al. 1998) were used for TEF1-α; CL1 and CL2a (O'Donnell et al. 2000) or CL3F and CL3R (De Beer et al. 2016) were used for CAL. PCR and sequencing protocols were as described by Jankowiak et al. (2019d), other than the annealing temperature being optimised for some individual reactions. All analyses were run independently for each gene partition (Figs 1–4). Resulting trees were visually compared for topological incongruence. Gene partitions showing no topological incongruence (βT, CAL) were combined and presented as a concatenated construct (Fig. 5).

For phylogenetic analyses, sequence alignments were performed using the online version of MAFFT v7 (Katoh and Standley 2013). The ITS, βT, CAL, and TEF1-α datasets were aligned using the E-INS-i strategy with a 200PAM/$\kappa$=2 scoring matrix, a gap opening penalty of 1.53 and an offset value of 0.00. The alignments were checked manually with BioEdit v.2.7.5 (Hall 1999). The resulting alignments and trees were deposited into TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S27966).

Phylogenetic trees were inferred for each of the datasets using three different methods: Maximum likelihood (ML), Maximum Parsimony (MP) and Bayesian inference (BI). For ML and BI analyses, the best-fit substitution models for each aligned dataset

were established using the corrected Akaike Information Criterion (AICc) in jModelTest 2.1.10 (Guindon and Gascuel 2003; Darriba et al. 2012). ML analyses were carried out with PhyML 3.0 (Guindon et al. 2010), utilizing the Montpelier online server (http://www.atgc-montpellier.fr/phyml/). The ML analysis included bootstrap analysis (1000 bootstrap pseudoreplicates) in order to assess node support values and the overall reliability of the tree topology. The best evolutionary substitution model was GTR+I+G for ITS (-lnL = 4497.47), GTR+G for CAL (-lnL = 4112.25) and TEF1-α (-lnL = 4218.36), HKY+G for βT (-lnL = 2641.05) and HKY+I+G for combined βT-CAL (-lnL 6798.48).

MP analyses were performed using PAUP* 4.0b10 (Swofford 2003). Gaps were treated as fifth state. Bootstrap analysis (1000 bootstrap replicates) was conducted to determine the levels of confidence for the nodes within the inferred tree topologies. Tree bisection and reconnection (TBR) was selected as the branch swapping option. The tree length (TL), Consistency Index (CI), Retention Index (RI), Homoplasy Index (HI) and Rescaled Consistency Index (RC) were recorded for each analysed dataset after the trees were generated.

BI analyses using Markov Chain Monte Carlo (MCMC) methods were carried out with MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003). Four MCMC chains were run for 10 million generations applying the best-fit model for each dataset. Trees were sampled every 100 generations, resulting in 100,000 trees. Tracer v1.4.1 (Rambaut and Drummond 2007) was utilized to determine the burn-in value for each dataset. The remaining trees were utilised to generate a 50% majority rule consensus tree, which allowed for calculating posterior probability values for the nodes.

## Results

### Phylogenetic Analyses

Alignments for the ITS dataset contained 575 characters; for the βT 303 characters; for CAL 543 characters; and for TEF1-α 812 characters; for the concatenated combined dataset 826 (including gaps), of which respectively 202, 123, 271, 439, 390 were parsimony-informative. The exon/intron arrangement of the βT data included exons 5 and 6, interrupted by intron 5. The exon/intron arrangement of the CAL data included exons 4 and 5, interrupted by intron 4. The aligned TEF1-α gene region consisted of intron 3 and exons 4 and 5, but lacked intron 4.

DNA sequence data were generated for 24 isolates considered in this study (Table 1). Blast analyses of the ribosomal DNA sequences placed all the isolates in *Sporothrix*. Based on phylogenetic analyses of the ITS (Fig. 1), the isolates emerged as six undescribed taxa. Phylogenetic analysis of the ITS indicated that the unknown species resided in two previously defined *Sporothrix* species complexes, including the *S. gossypina*- and *S. stenoceras*- species complexes, and lineage "F". Additionally, isolates representing two new species grouped outside any of the currently defined species

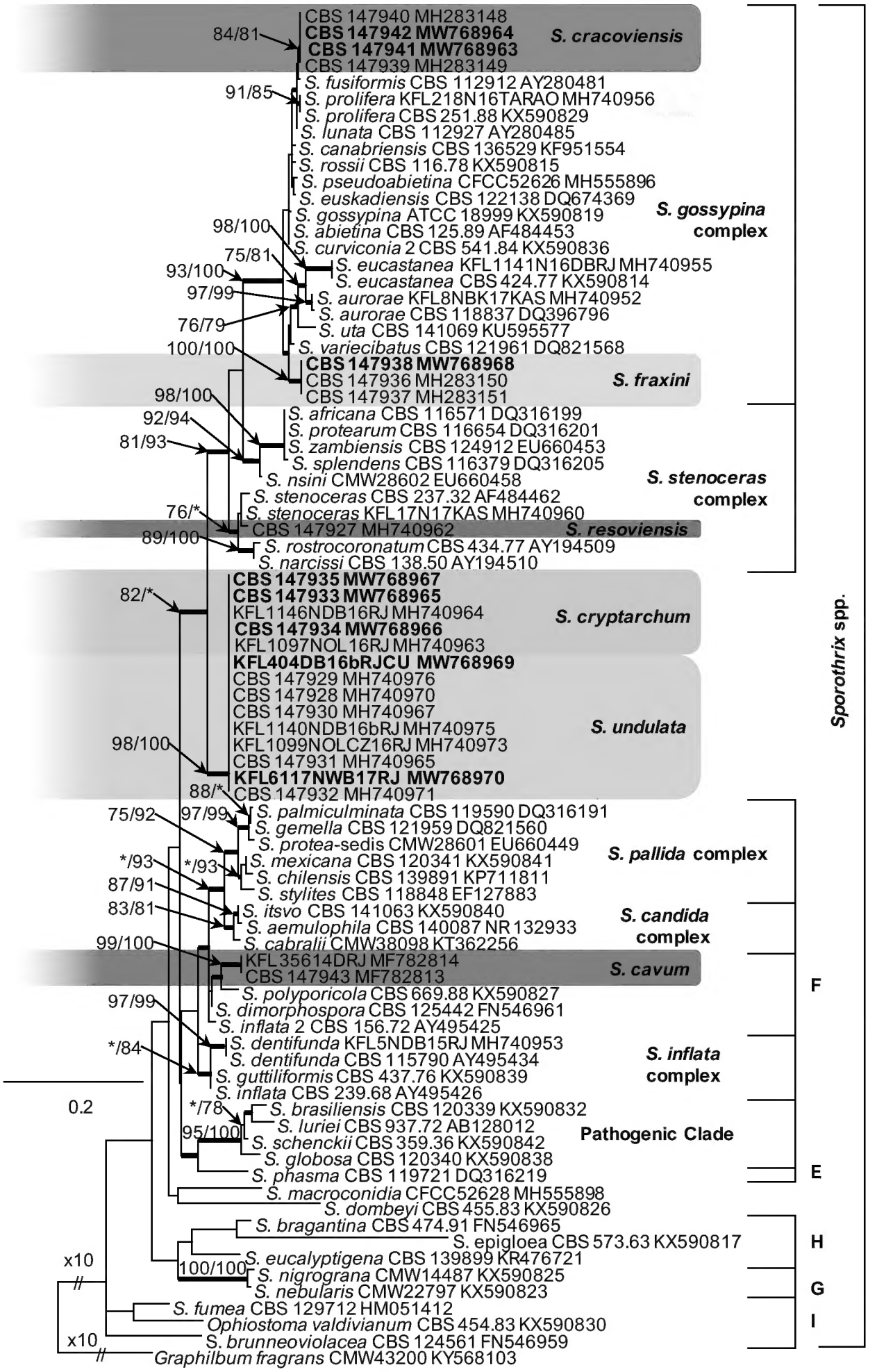


**Figure 1.** Phylogram obtained from Maximum Likelihood (ML) analyses of the ITS1-5.8S-ITS2 data for the *Sporothrix* spp. Sequences obtained during this study are presented in bold type. The Bootstrap values ≥ 75% for ML and Maximum Parsimony (MP) analyses are presented at nodes as follows: ML/MP. Bold branches indicate posterior probabilities values ≥ 0.95 obtained from Bayesian Inference (BI) analyses. * Bootstrap values <75%. The tree is drawn to scale (see bar) with branch length measured in the number of substitutions per site. *Graphilbum fragrans* represent the outgroup.

complexes (Fig. 1). Based on the availability of sequence data for these complexes, different datasets were assembled and analysed separately for each species complex.

Seven isolates from hardwood-infesting bark beetles identified as *Sporothrix* 7 and *Sporothrix* 8 by Jankowiak et al. (2019a) resided in the *S. gossypina*-complex (Fig. 1). All three gene regions (ITS, βt, CAL) separated *Sporothrix* sp. 8 from the other known species with strong statistical support (Figs 2–4). The ITS and βt gene regions grouped isolates of this species together with the ex- type isolate of *S. variecibatus*, while CAL gene region placed it with *S. aurorae* (Figs 1–3). Isolates representing *Sporothrix* sp. 7 had ITS sequences that were almost identical to the ITS sequences for *S. fusiformis*, *S. lunata* and *S. prolifera* (Fig. 1). In the βt and CAL trees (Figs 2, 3), *Sporothrix* sp. 7 formed lineages that clearly separated this species from the known species in the *S. gossypina* complex, and although there were differences in the βt sequence compared to other species, the node lacked statistical support (Fig. 2). The combined analyses of the βt and CAL datasets clearly distinguish *Sporothrix* sp. 7 and *Sporothrix* sp. 8 into separate lineages within the *S. gossypina*-complex (Fig. 5).

The single isolate from a wound on *Betula pendula* identified as *Sporothrix* sp. 10 by Jankowiak et al. (2019b), resided in *S. stenoceras*-complex and grouped closely with *S. stenoceras* sensu stricto based on analysis of ITS, βt, CAL, and TEF1-α gene regions (Figs 1–4). All three gene regions separated *Sporothrix* sp. 10 from *S. stenoceras*, although this separation was not statistically supported by the ITS gene region (Figs 1–4). The combined analyses of the βt and CAL datasets clearly distinguish *Sporothrix* sp. 10 into separate lineages within the *S. stenoceras*-complex (Fig. 5).

Two isolates from woodpecker cavities identified as *Sporothrix* sp. 18 by Jankowiak et al. (2019c), belonged to the lineage F defined by De Beer et al. (2016) based on the ITS tree. All the three gene regions (ITS, βt, CAL) separated *Sporothrix* sp. 18 from the other known species in lineage F with strong statistical support (Figs 1–4). The combined analyses of the βt and CAL datasets clearly distinguish *Sporothrix* sp. 18 into separate lineages within the *Sporothrix* spp. (Fig. 5).

Fourteen isolates from wounds on different species of hardwood trees and nitidulid beetles identified as *Sporothrix* sp. 11 and *Sporothrix* sp. 12 by Jankowiak et al. (2019b) did not group in any of the defined *Sprothrix* species complexes based on analysis of ITS gene region and formed a monophyletic lineage within *Sporothrix* (Fig. 1). Isolates of *Sporothrix* sp. 11 had ITS sequences that were identical with ITS sequences noted in *Sporothrix* sp. 12. In the βt, CAL, and TEF1-α trees (Figs 2–4), *Sporothrix* sp. 11 and *Sporothrix* sp. 12 formed well-supported lineages that clearly separated these two putative new species from each other. The combined analyses of the βt and CAL datasets also separated *Sporothrix* sp. 11 and Sporothrix sp. 12 from the other known species in *Sporothrix* spp. and also from each other (Fig. 5).

## Morphological characteristics

The six new taxa in *Sporothrix* emerging from the phylogenetic studies showed differences in colony colour. The cultures of *Sporothrix* spp. 7, 8, 10 and 11 were white.

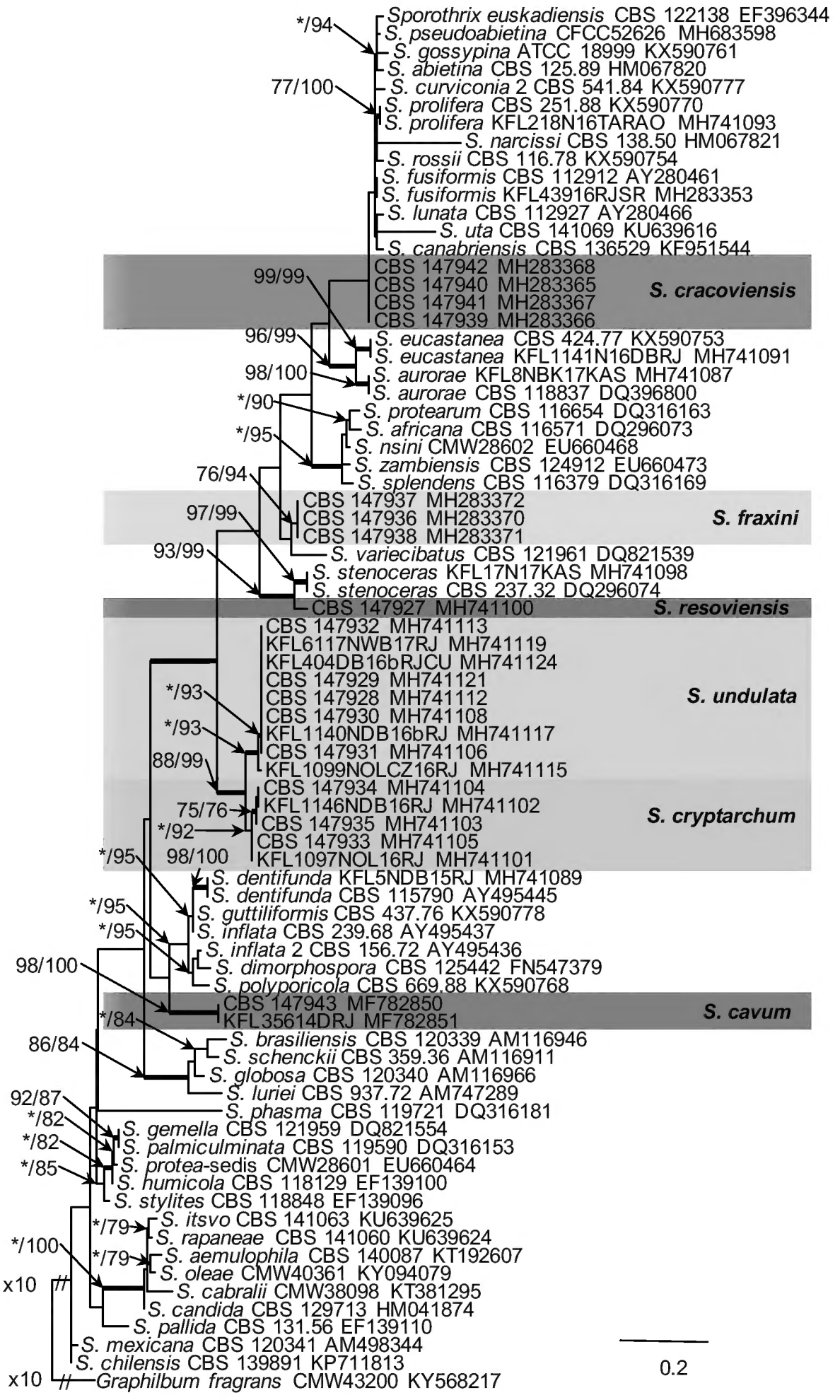


**Figure 2.** Phylogram obtained from Maximum Likelihood (ML) analyses of βT data for the *Sporothrix* spp. Sequences obtained during this study are presented in bold type. The Bootstrap values ≥ 75% for ML and Maximum Parsimony (MP) analyses are presented at nodes as follows: ML/MP. Bold branches indicate posterior probabilities values ≥ 0.95 obtained from Bayesian Inference (BI) analyses. * Bootstrap values <75%. The tree is drawn to scale (see bar) with branch length measured in the number of substitutions per site. *Graphilbum fragrans* represent the outgroup.

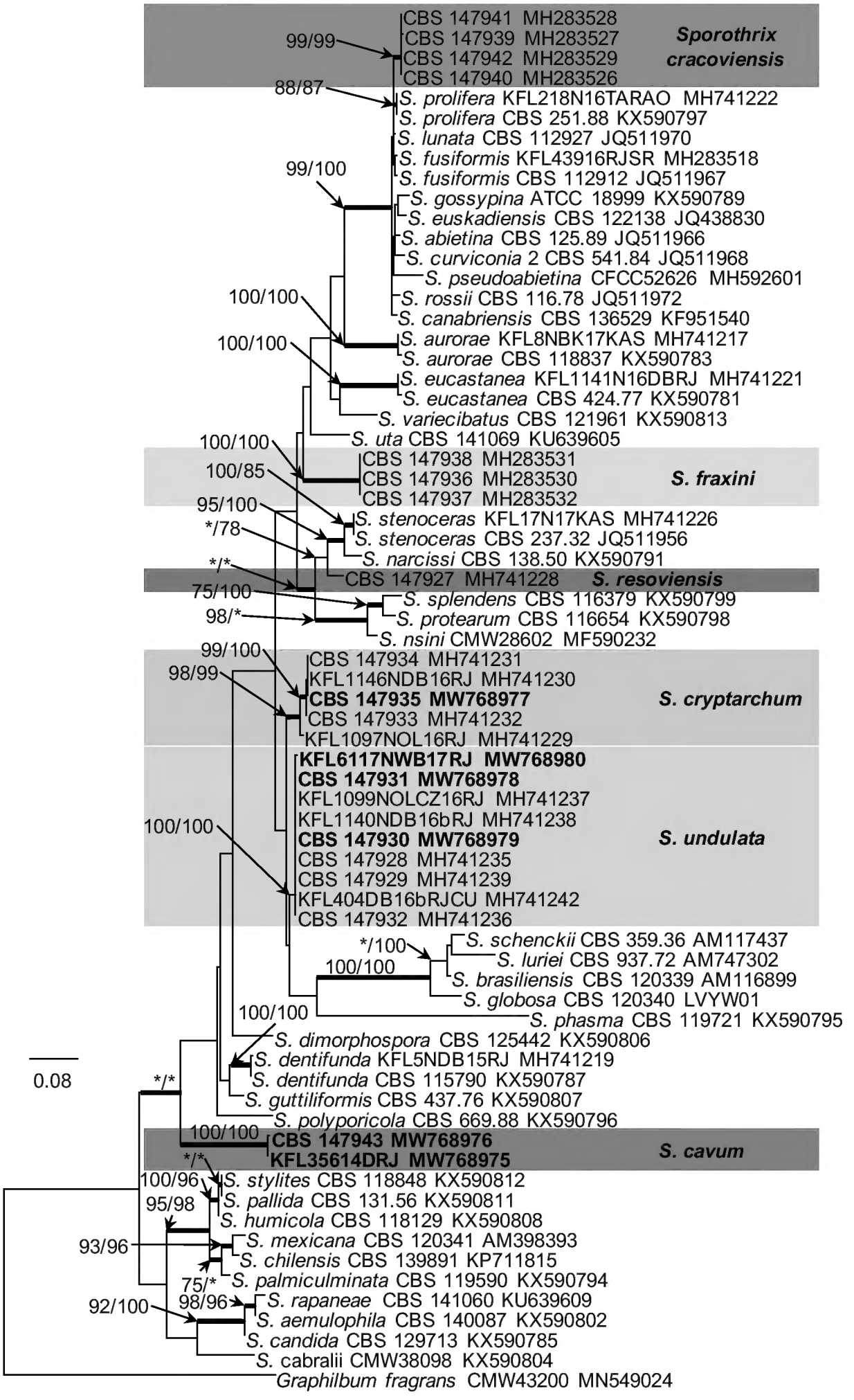


**Figure 3.** Phylogram obtained from Maximum Likelihood (ML) analyses of CAL data for the *Sporothrix* spp. Sequences obtained during this study are presented in bold type. The Bootstrap values ≥ 75% for ML and Maximum Parsimony (MP) analyses are presented at nodes as follows: ML/MP. Bold branches indicate posterior probabilities values ≥ 0.95 obtained from Bayesian Inference (BI) analyses. * Bootstrap values <75%. The tree is drawn to scale (see bar) with branch length measured in the number of substitutions per site. *Graphilbum fragrans* represent the outgroup.

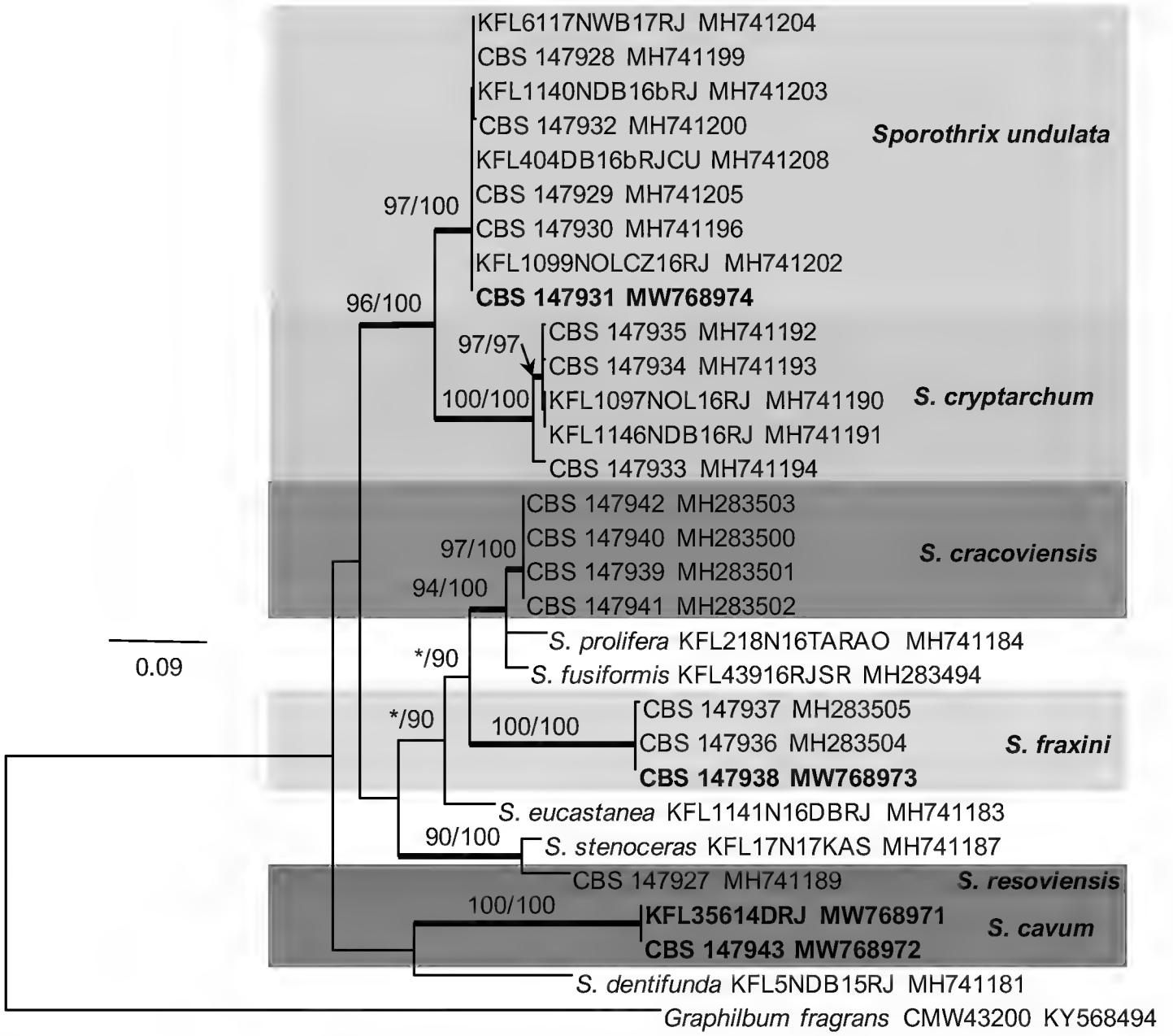


**Figure 4.** Phylogram obtained from Maximum Likelihood (ML) analyses of TEF1-α data for the *Sporothrix* spp. Sequences obtained during this study are presented in bold type. The Bootstrap values ≥ 75% for ML and Maximum Parsimony (MP) analyses are presented at nodes as follows: ML/MP. Bold branches indicate posterior probabilities values ≥ 0.95 obtained from Bayesian Inference (BI) analyses. * Bootstrap values <75%. The tree is drawn to scale (see bar) with branch length measured in the number of substitutions per site. *Graphilbum fragrans* represent the outgroup.

The cultures of *Sporothrix* sp. 12 were white or pigmented (white grey) whereas cultures of *Sporothrix* sp. 18 were greyish green. With the exception of *Sporothrix* sp. 7 cultures that had an optimum growth at 25 °C followed by 20 °C, all of the undescribed taxa displayed optimum growth at 25 °C followed by 30 °C.

All the new taxa emerging from this study produced micronematous conidiophores and hyaline or pigmented conidia formed holoblastically on denticulate conidiogenous cells. *Sporothrix* sp. 11 and *Sporothrix* sp. 12 were characterized by the formation of hyaline and pigmented conidia. Other than *Sporothrix* sp. 18, which remained asexual, a sexual morph was induced in all five of the other emerging taxa. Ascomata were black and globose with straight necks and up to 700 µm long. Ostiolar hyphae were well-developed and up to 74 µm long. Ascospores were allantoid (*Sporothrix* sp. 7, 8) or kidney-shaped (*Sporothrix* spp. 10–12), and they lacked sheaths.

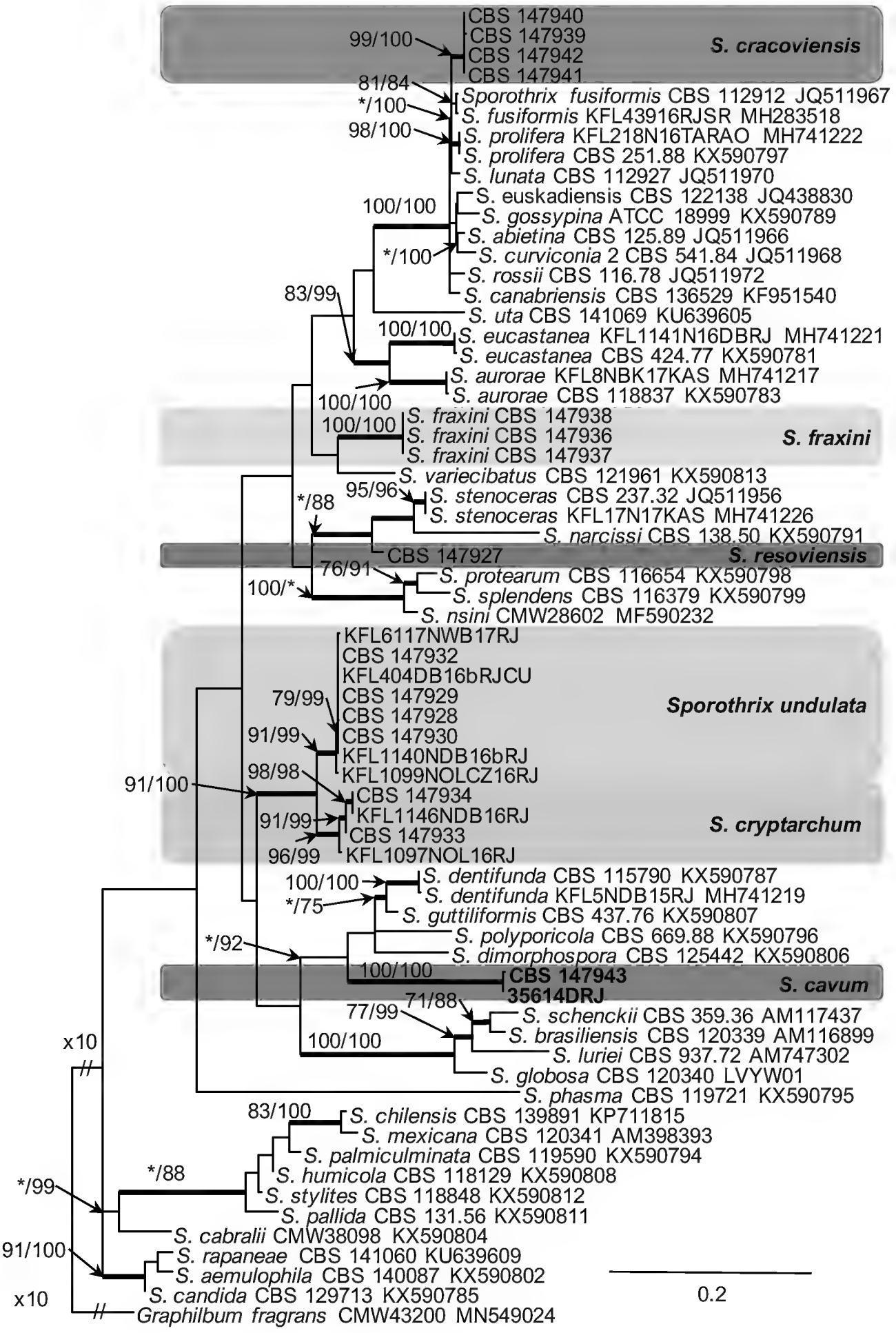


**Figure 5.** Phylogram obtained from Maximum Likelihood (ML) analyses of the combined βT and CAL sequences of the *Sporothrix* spp. Sequences obtained during this study are presented in bold type. The Bootstrap values ≥ 75% for ML and Maximum Parsimony (MP) analyses are presented at nodes as follows: ML/MP. Bold branches indicate posterior probabilities values ≥ 0.95 obtained from Bayesian Inference (BI) analyses. * Bootstrap values <75%. The tree is drawn to scale (see bar) with branch length measured in the number of substitutions per site. *Graphilbum fragrans* represent the outgroup.

## Taxonomy

***Sporothrix cracoviensis* R. Jankowiak, sp. nov.**
MycoBank No: 840460
Fig. 6

**Etymology.** From Latin, referring to the capital of Małopolskie Voivodeship and the former capital of Poland (Cracovia in Latin, Kraków in Polish); the region where this fungus was collected.

**Type.** Poland, Małopolskie Province, Krzeszowice, from adult *Tryopodendron domesticum* beetle on *Fagus sylvaticum*, January 2014, *R. Jankowiak* (O-F-258628 ***holotype***, culture ex-type CBS 147942).

**Description.** Sexual and asexual structures produced on sterilised beech twigs on surface of malt agar in Petri dishes. *Ascomata* abundant, superficially or partly embedded in the agar, single or in groups; *ascomatal bases* black, globose, (66–)89–153(–245) µm diam., with brown hyphal hairs, 12 to 165 µm long and 1 to 1.8 µm wide at the base; *ascomatal necks* black, straight or curved, (187–)272–462(–611) µm long, diameter (9–)10.4–16.7(22.5) µm at the apex and (26.8–)29.9–50.5(–63.9) µm at the base. *Ostiolar hyphae* present, pale brown, septate, straight or slightly waved, tapering towards the apex or sporadically dichotomous branching at the tip, (7–)8–16(–22) in number (17.8–)29.6–48.4(–64.5) µm long, (0.3–)0.5–1(–1.5) µm at the apex and (1.2–)1.6–2.3–(3) µm at the base. *Asci* evanescent. *Ascospores* one-celled, allantoid in side view (2.8–)3.1–3.8(–5.1) × (1–)1.1–1.4(–1.6) µm, elliptical in front view (2.8–)3.1–4.2(–4.8) × (1–)1.2–1.5(–1.8) µm, sometimes with residual sheath up to 1 µm thick, accumulated in creamy-colored mass at the tip of the neck. *Conidiophores* hyaline, micronematous, simple or branched, straight, simple or branched, bearing several conidiogenous cells, either borne on vegetative hyphae or on upright hyphae. *Conidiogenous cells* blastics, cylindrical, terminal, lateral or intercalary, straight or curved, tapering towards the apex, swollen apical part forming conidia by sympodial proliferation on visible denticles, (4.2–)17.5–43.1(–72.2) µm long, (0.8–)1.1–1.7(–2.1) µm wide at the base. Apical part with denticles (0.8–)1.3–3.7(–7.3) µm long and (1.2–)1.7–3.7(–7.3) µm wide. Conidia hyaline, unicellular, smooth, obovoid to clavate, sometimes slightly curved, with slightly pointed bases, (2.8–)3.2–6.4(–8.7) × (1.1–)1.4–2.1(–2.7) µm, formed directly on denticles. *Culture characteristics*: Cultures showing optimal growth at 25 °C (1 mm/d) with somewhat slower growth by at 20 °C (0.8 mm/d), white, flat, floccose, growing in a circular pattern with smooth margins.

**Host tree.** *Fagus sylvatica*.

**Insect vector.** *Trypodendron domesticum*, *T. signatum*.

**Distribution.** Poland

**Additional specimen examined.** Poland, Małopolskie Province, Krzeszowice, from adult *Tryopodendron domesticum* beetle on *Fagus sylvaticum*, January 2014, *R. Jankowiak* (O-F-258629, cultures CBS 147941).

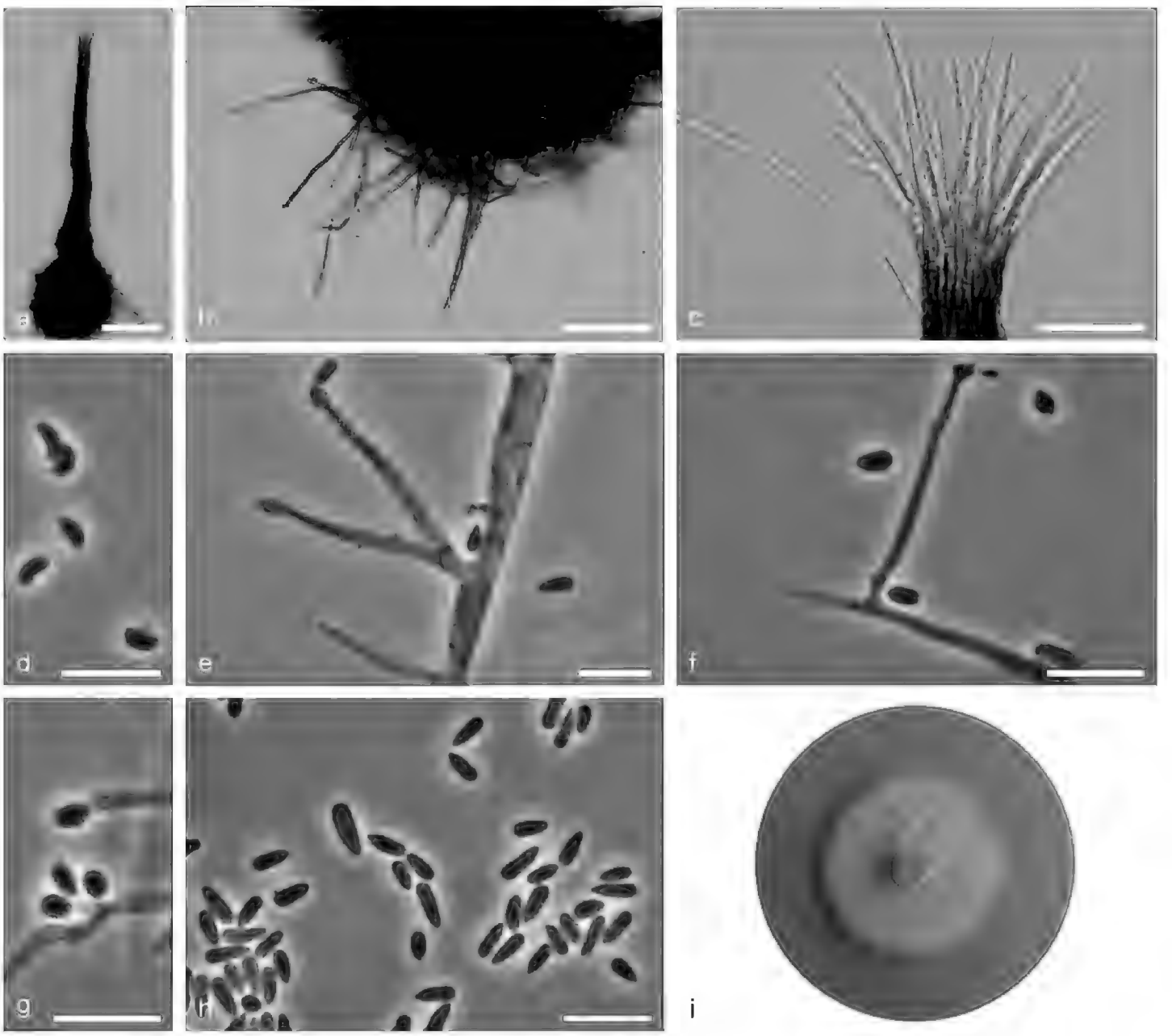

**Figure 6.** *Sporothrix cracoviensis* sp. nov. (CBS 147942) **a** ascoma **b** ascomatal base **c** ostiolar hyphae **d** ascospores **e, f** conidiogenous cell with an inflated cluster of denticles at the apex **g** conidiogenous cells arising directly from hyphae **h** conidia **i** fourteen-day-old culture on MEA. Scale bars: 50 μm (**a, b**), 25 μm (**c**), 10 μm (**d–h**).

**Notes.** *Sporothrix cracoviensis* is phylogenetically distinct from the other *Sporothrix* species based on the βT, CAL and TEF1-α sequences. This species is closely related to *S. fusiformis*, *S. lunata* and *S. prolifera*. *Sporothrix cracoviensis* has smaller ascomatal necks (187–611 μm) compared to *S. fusiformis* (301–1168) μm (Aghayeva et al. 2004). Their conidial dimensions and shapes showed also differences. *Sporothrix fusiforme* has fusiforme conidia (Aghayeva et al. 2004), whereas *S. cracoviensis* has obovoid to clavate conidia. *Sporothix lunata* has also different shape of conidia (crescent) (Aghayeva et al. 2004) compared to *S. cracoviensis* (obovoid to clavate). In addition, *S. lunata* has smaller conidia (2.3–6.2 × 0.8–1.6 μm) (Aghayeva et al. 2004) compared to *S. cracoviensis* (2.8–8.7 μm × 1.1–2.7 μm). *Sporothrix prolifera* could be distinguished from *S. cracoviensis* by its smaller ascomatal base (*S. prolifera*: 65–90 μm (Kowalski and Butin 1989); *S. cracoviensis*: 66–245 μm) and smaller ascomatal necks (*S. prolifera*: 75–160 μm (Kowalski and Butin 1989); *S. cracoviensis*: 187–611 μm). In addition,

*S. prolifera* has shorter ostiolar hyphae (*S. prolifera*: 15–30 µm (Kowalski and Butin 1989); *S. cracoviensis*: 26.8–63.9 µm) and shorter and wider ascospores (*S. prolifera*: 3.2–3.8 × 1.8–2 µm (Kowalski and Butin 1989); *S. cracoviensis*: 2.8–5.1 × 1–1.6 µm). The conidia of *S. prolifera* are also smaller (*S. prolifera*: 4–5.8 × 1.6–2.2 µm (Kowalski and Butin 1989) compared to *S. cracoviensis* (2.8–8.7 × 1.1–2.7 µm).

*Sporothrix cracoviensis* was represented by four isolates collected from adult *Trypodendron domesticum* beetles on *Fagus sylvatica*. It corresponds to *Sporothrix* sp. 7 in the study of Jankowiak et al. (2019a).

***Sporothrix fraxini* R. Jankowiak, sp. nov.**
MycoBank No: 840463
Fig. 7

**Etymology.** From Latin, referring to the genus name of the host (*Fraxinus excelsior*).

**Type.** Poland, Małopolskie Province, Zbylitowska Góra, from the gallery of *Hylesinus varius* on *Fraxinus excelsior*, May 2016, *R. Jankowiak* (O-F-258630 ***holotype***, culture ex-type CBS 147936).

**Description.** Sexual and asexual structures produced on sterilized ash twigs and on surface of malt agar in Petri dishes. *Ascomata* abundant, superficially or partly embedded in the agar, single or in groups; *ascomatal base* black, globose, (89–)110–161(–216) µm diam., with brown hyphal hairs, 14 to 65 µm long and 1.1 to 2.1 µm wide at the base; *ascomatal necks* black, straight or curved, (222–)332–461(–526) µm long, diameter (10.1–)11.3–16(–20.4) µm at the apex and (26.2–)29.1–41.4(–53) µm at the base. *Ostiolar hyphae* present, pale brown, septate, straight or rather tortuous, tapering towards the apex or sporadically dichotomous branching at the tip, (8–)10–20(–24) in number (21.4–)31.1–52.1(–73.6) µm long, (0.4–)0.7–1.1(–1.4) µm at the apex and (1.4–)1.8–2.4–(3.1) µm at the base. *Asci* evanescent. *Ascospores* one-celled, allantoid in side view (2.7–)2.9–3.5(–4.4) × (0.9–)1–1.4(–1.8) µm, elliptical in front view (2.2–)2.9–3.8(–4.7) × (0.8–)1.2–1.6(–1.8) µm, sometimes with residual sheath up to 1 µm thick, accumulated in white-color mass at the tip of the neck. *Conidiophores* hyaline, micronematous, simple or branched, straight, simple or branched, bearing several conidiogenous cells, either borne on vegetative hyphae or on upright hyphae. *Conidiogenous cells* blastic, cylindrical terminal or intercalary, straight or curved, tapering towards the apex, swollen apical part forming conidia by sympodial proliferation on hardly visible denticles, (13.6–)14.6–47.7(–99.6) µm long, (0.9–)1.2–1.6(–1.9) µm wide at the base. Apical part (0.8–)1.7–5.1(–10.6) µm long and (0.8–)1.1–2(–3) µm wide. *Conidia* hyaline, unicellular, smooth, obovoid to ellipsoidal, ends slightly rounded or truncate, (2.6–)3.4–5(–6.8) × (0.8–)1.1–1.6(–2) µm, formed directly on denticles. *Culture characteristics*: Cultures showing optimum growth at 25 °C (1 mm/d) followed by at 30 °C (0.9 mm/d), white, flat, growing in a circular pattern with smooth margins, with sparse aerial mycelium, often fading around the edges.

**Host tree.** *Fraxinus excelsior*.

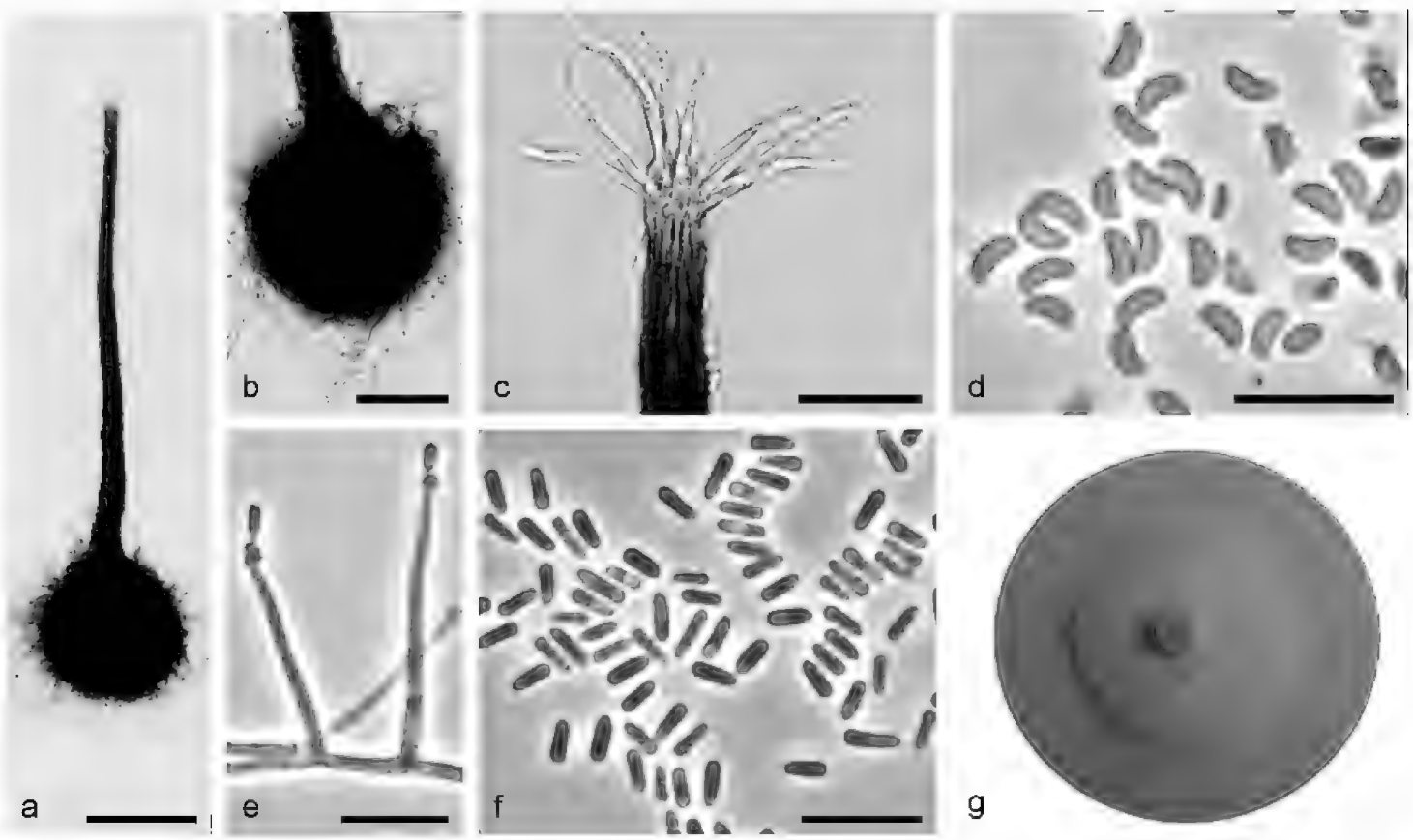


**Figure 7.** *Sporothrix fraxini* sp. nov. (CBS 147936) **a** ascoma **b** ascomatal base **c** ostiolar hyphae **d** ascospores **e** conidiogenous cell with an inflated cluster of denticles at the apex **f** conidia **g** fourteen-day-old culture on MEA. Scale bars: 100 µm (**a**), 50 µm (**b**), 25 µm (**c**), 10 µm (**d–f**).

**Insect vector.** *Hylesinus crenatus*, *H. varius*.

**Distribution.** Poland

**Additional specimen examined.** Poland, Małopolskie Province, Zbylitowska Góra, from the gallery of *Hylesinus varius* on *Fraxinus excelsior*, May 2016, *R. Jankowiak* (O-F-258631, cultures CBS 147938).

**Notes.** This species is phylogenetically distinct from the other *Sporothrix* species based on the ITS, βT, CAL and TEF1-α sequences. *Sporothrix fraxini* is closely related to *S. variecibatus*. However, *S. variecibatus* does not produce a sexual morph, and has narrower conidia (2–3 µm) (Roets et al. 2008) compared to *S. fraxini* (0.8–2 µm). In addition, the conidia of *S. variecibatus* are clavate while *S. fraxini* has obovoid to ellipsoidal conidia.

*Sporothrix fraxini* was represented by three isolates collected from the galleries of *Hylesinus varius* on *Fraxinus excelsior*. It corresponds to *Sporothrix* sp. 8 in the previous study of Jankowiak et al. (2019a).

***Sporothrix resoviensis* R. Jankowiak & A. Ostafińska, sp. nov.**

MycoBank No: 840475

Fig. 8

**Etymology.** From Latin, referring to the capital of Podkarpackie Voivodeship (Resovia in Latin, Rzeszów in Polish), the region from which this fungus was collected.

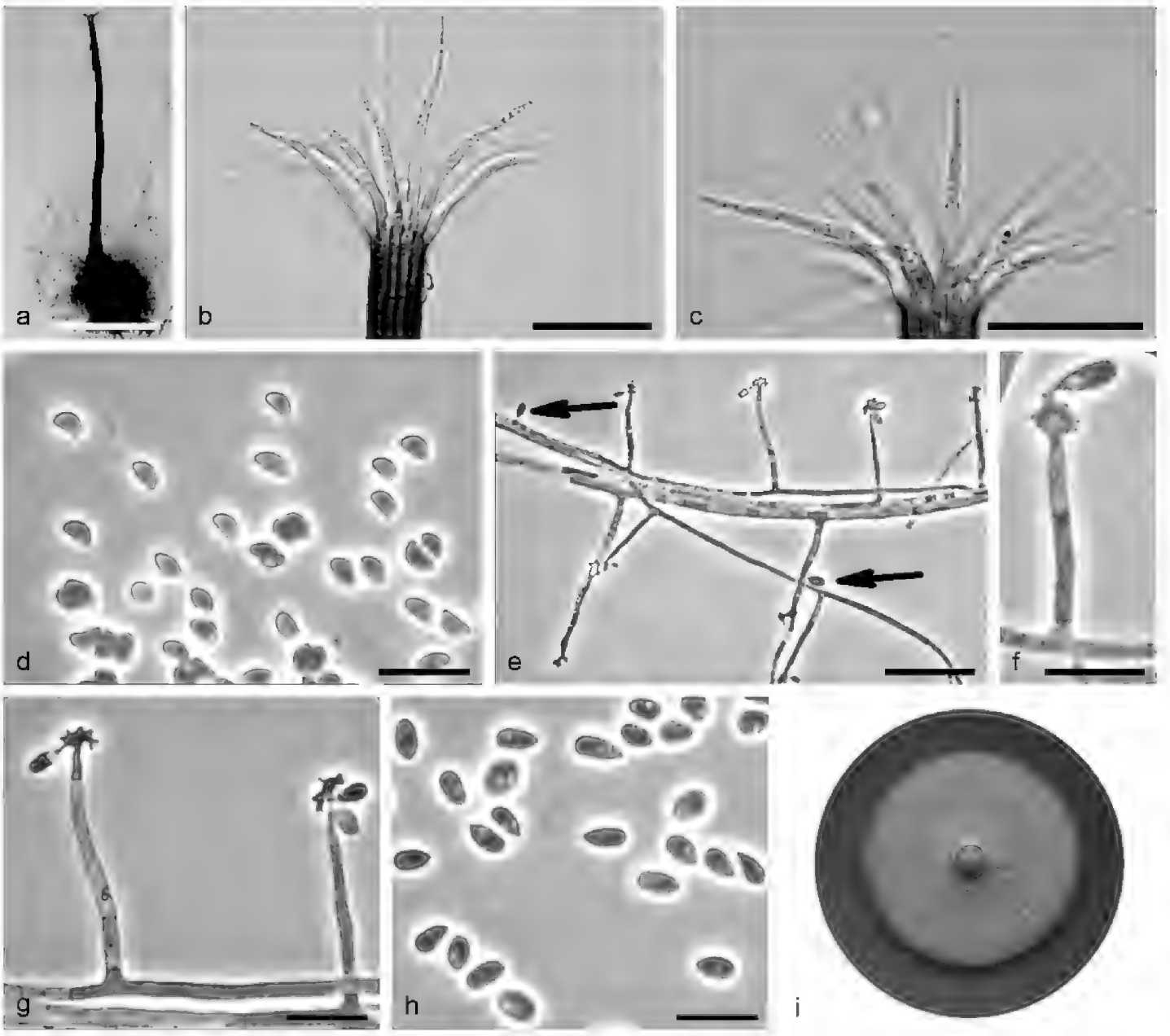


**Figure 8.** *Sporothrix resoviensis* sp. nov. (CBS 147927) **a** ascoma **b, c** ostiolar hyphae **d** ascospores **e–g** conidiogenous cell with an inflated cluster of denticles at the apex **h** conidia **i** fourteen-day-old culture on MEA. Scale bars: 250 µm (**a**), 25 µm (**b, c**), 10 µm (**d**), 25 µm (**e**), 10 µm (**f–h**).

**Type.** Poland, Podkarpackie Province, Borownica, from the wound on *Betula pendula*, June 2016, *A. Ostafińska*, (O-F-258632 ***holotype***, culture ex-type CBS 147927).

**Description.** Sexual and asexual structures produced on sterilised birch twigs and on surface of malt agar in Petri dishes. *Ascomata* abundant, superficially or partly embedded in the agar, single or in groups; *ascomatal bases* black, globose, (87–)113–184(–232) µm diam., with brown hyphal hairs, 14 to 44 µm long and 0.9 to 2.2 µm wide at the base; *ascomatal necks* black, straight or curved, often extended at the base, (228–)378–624(–700) µm long, diameter (10–)11.2–17(–20.2) µm at the apex and (26.2–)34–47.7(–56) µm at the base. *Ostiolar hyphae* present, pale brown, septate, straight or curved, tapering towards the apex and often swollen at the tip, (7–)9–15(–18) in number, (15.7–)26.1–47.7(–67.6) µm long, (0.3–)0.7–1.5(–2.5) µm at the apex and (1.3–)2–3–(3.4) µm at the base. *Asci* evanescent. *Ascospores* one-celled, kidney-shaped to almost triangular in side view (2.7–)3.2–3.9(–4.4) × (1.4–)1.7–2.1(–2.3) µm, oblong-elliptical in front view (2.6–)3–3.8(–4.9) × (1.4–)1.7–2.2(–2.6) µm, without residual

sheath accumulated in white-colored mass at the tip of the neck. *Conidiophores* hyaline, micronematous, straight, simple and bearing several conidiogenous cells, either borne on vegetative hyphae or on upright hyphae. *Conidiogenous cells* blastic, cylindrical, terminal, lateral or intercalary, straight or curved, swollen apical part forming conidia by sympodial proliferation on easily visible denticles, (3.1–)9.3–57(–120.1) µm long, (1–)1.1–1.6(–2.2) µm wide at the base. Apical part (1.3–)1.9–3.5(–4.4) µm long and (1.4–)2.4–3.9(–4.5) µm wide. *Conidia* hyaline, unicellular, smooth, obovate to ellipsoidal, pointed at the base, (3.9–)4.3–6.7(–8.5) × (2.1–)2.4–3.4(–4) µm, formed singly on denticles or on the side of vegetative hyphae. *Culture characteristics*: Cultures showing optimum growth at 25 °C (1.8 mm/d) followed by at 30 °C (1.7 mm/d), white, growing in a circular pattern with smooth margins, funiculose and woolly.

**Host trees.** *Betula pendula*.

**Insect vector.** unknown.

**Distribution.** Poland.

**Note.** *Sporothrix resoviensis* is phylogenetically distinct from the other *Sporothrix* species based on the ITS, βT, CAL and TEF1-α sequences. This species grouped most closely with *S. stenoceras* but can be distinguished by its larger ascospores (*S. resoviensis*: 2.7–4.4 × 1.4–3.3 µm; *S. stenoceras*: 2.0–2.9 × 1.3–1.4 µm (Robak 1932). Perithecia developing on the agar medium and twigs have significantly shorter necks (*S. resoviensis*: 228–700 µm; *S. stenoceras*: 450–1500 µm (Robak 1932). *Sporothrix resoviensis* has larger conidia (3.9–8.5 × 2.1–4 µm) compared to *S. stenoceras* (3.4–6.9 × 2–3.4 µm). This new species also differs from *S. stenoceras* based on culture morphology, where *S. resoviensis* produces wooly cultures, different to the sparse and flat mycelium of *S. stenoceras* (Robak 1932).

*Sporothrix resoviensis* was represented by one isolate collected from a wound on *Betula pendula*. It corresponds to *Sporothrix* sp. 10 in the study of Jankowiak et al. (2019b).

***Sporothrix cryptarchum* R. Jankowiak & A. Ostafińska, sp. nov.**

MycoBank No: 840477

Fig. 9

**Etymology.** Referring to the genus name of the beetle, *Cryptarcha* sp. (*Coleoptera*: *Nitidulidae*), with which this fungus is associated.

**Type.** Poland, Małopolskie Province, Wierzchosławice, from *Cryptarcha undata* on *Quercus robur*, June 2016, *R. Jankowiak*, (O-F-258633 ***holotype***, culture ex-type CBS 147934).

**Description.** Sexual and asexual structures produced on the sterilised oak twigs and on the surface of malt agar in Petri dishes. *Ascomata* abundant, superficially or partly embedded in the agar, single or in groups; *ascomatal bases* black, globose, (55–)115–172(–210) µm diam., with brown hyphal hairs, 15 to 141 µm long and 0.9 to 3.8 µm wide at the base; *ascomatal necks* black, straight or curved, (126–)198–

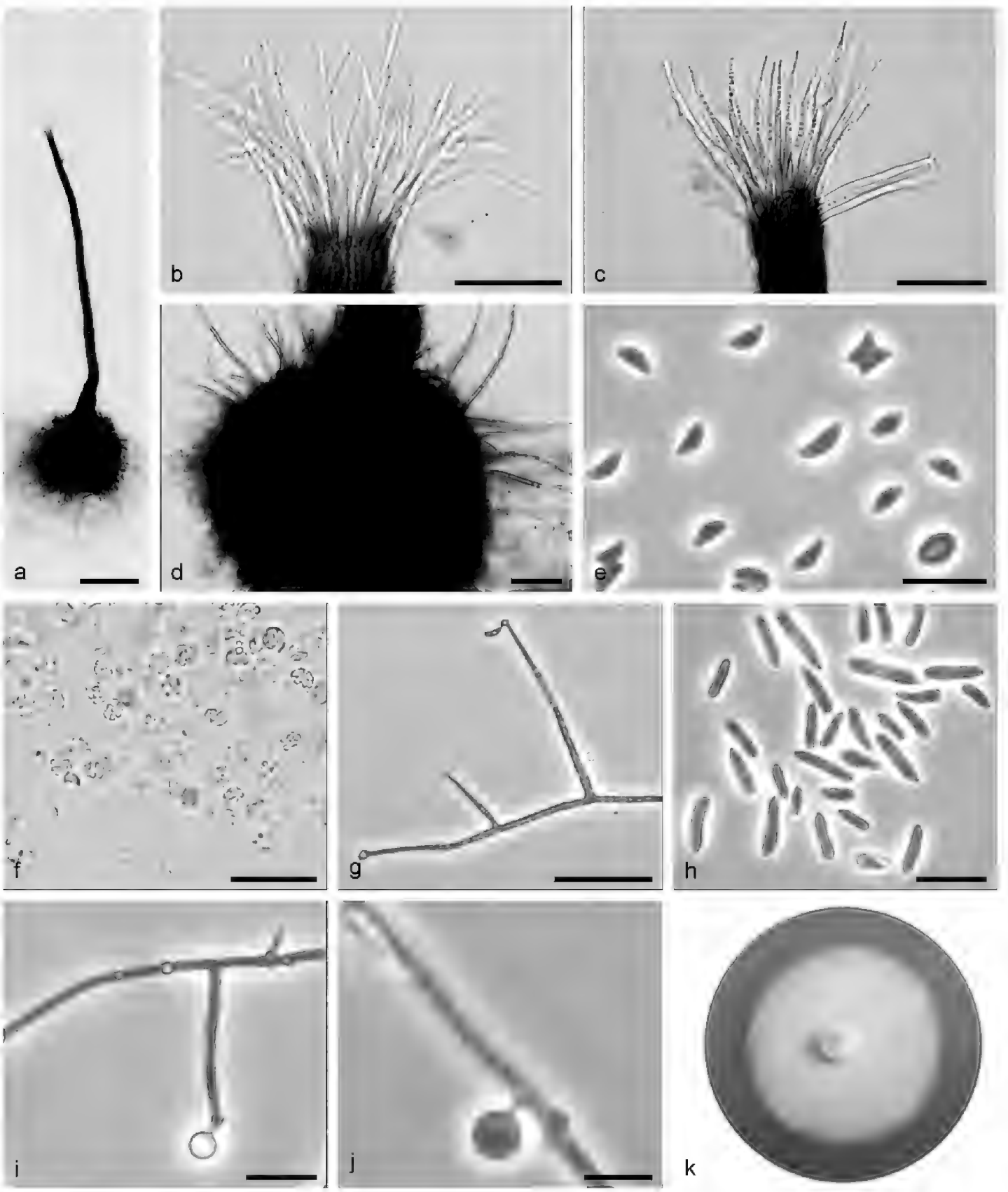


**Figure 9.** *Sporothrix cryptarchum* sp. nov. (CBS 147934) **a** ascoma **b** ascomatal base **c, d** ostiolar hyphae **e** ascospores **f** asci **g** conidiogenous cell with an inflated cluster of denticles at the apex **h** conidia **i** globose conidia arising on long conidiophore **j** globose conidia arising directly from hyphae **k** fourteen-day-old culture on MEA. Scale bars: 100 µm (**a**), 25 µm (**b–d**), 10 µm (**e**), 25 µm (**f, g**), 10 µm (**h, i**), 5 µm (**j**).

412(–544) µm long, diameter (10.9–)13–19(–23.8) µm at the apex and (17.6–)29.3–47.6(–59.6) µm at the base. *Ostiolar hyphae* present, pale brown, with small granules, septate, straight or curved, simple or dichotomous branching, tips tapering or sometimes thickened, (9–)13–24(–31) in number, (15.8–)30.5–51.8(–60.9) µm long, (0.2–)0.3–0.5(–0.7) µm at the apex and (0.9–)1.6–2.4–(3) µm at the base. *Asci* subglobose to

ovoid, (5.5–)6.7–9(–11) × (4–)4.9–6.2(–7.2) µm. *Ascospores* one-celled, kidney-shaped to almost triangular in side view in side view (3.2–)3.8–4.7(–5.8) × (0.8–)1–1.3(–1.5) µm, elliptical in front view (3.1–)3.6–4.4(–5) × (1–)1.2–1.6(–1.8) µm, sometimes with residual sheath up to 0.6 µm thick, accumulated in white-colored mass at the tip of the neck. *Conidiophores* hyaline, micronematous, simple or occasionally branched and bearing several conidiogenous cells, either borne on vegetative hyphae or on upright hyphae. *Conidiogenous cells* blastic, cylindrical, terminal, lateral or intercalary, straight or curved, tapering towards the apex, swollen apical part forming conidia by sympodial proliferation on narrow denticles, (2.2–)13.9–51.2(–102.8) µm long, (0.7–)1.2–1.8(–2.2) µm wide at the base. Apical part (0.6–)1.4–3.1(–5.3) µm long and (1–)1.7–3(–3.8) µm wide, single denticles often below. Conidia of two types: 1) abundant in cultures, often produced, hyaline, unicellular, smooth, obovate to ellipsoid, pointed at the base, (3.3–)4.6–8.1(–10.3) × (1–)1.3–1.9(–2.2) µm, formed directly on denticles; 2) sparse in cultures, subhyaline to lightly pigmented, unicellular, smooth, subglobose to globose, (2.3–)3.1–4.1(–4.5) µm diam, formed singly, either directly on the side of vegetative hyphae or on short lateral branches. *Culture characteristics*: Cultures showing optimum growth at 25 °C (1.3 mm/d) followed by at 30 °C (1.1 mm/d), mostly pigmented or white or pig, flat, growing in a circular pattern with smooth margins.

**Host tree.** *Alnus glutinosa*, *Quercus robur*.

**Insect vector.** *Cryptarcha undata*, *C. strigata*.

**Distribution.** Poland.

**Additional specimen examined.** Poland, Małopolskie Province, Wierzchosławice, from *Cryptarcha undata* on *Quercus robur*, June 2016, *R. Jankowiak*, (O-F-258634, cultures CBS 147933).

**Notes.** This species is phylogenetically distinct from the other *Sporothrix* species based on the ITS, βT, CAL and TEF1-α sequences. *Sporothrix cryptarchum* is phylogenetically closely related to *S. undulata* (*Sporothrix* sp. 12) described in the present study. This species also shares morphological similarities such as kidney-shaped ascospores and two morphological forms of conidia with *S. undulata*. However, *S. cryptarchum* has narrow ascospores (0.8–1.5 µm) compared to *S. undulata* (1.1–2 µm). It also has distinct ostiolar hyphae, with those in *S. cryptarchum* often dichotomously branching while in *S. undulata* these hyphae occur only sporadically and do not have dichotomous branching. Both species produce hyaline and pigmented conidia. However, *S. cryptarchum* cultures are predominantly hyaline whereas those in pure cultures of *S. undulata* are mostly pigmented. Their conidial shapes in these two species are similar but their dimensions are distinct. *Sporothrix cryptarchum* has conidia that are smaller than those of *S. undulata*. In addition, cultures of *S. cryptarchum* are white and grow in a circular pattern with smooth margins while those of *S. undulata* grow in a circular pattern with undulate margins and some have grey pigmentation.

*Sporothrix cryptarchum* was represented by four isolates collected from Poland. It corresponds to *Sporothrix* sp. 11 in the study of Jankowiak et al. (2019b). *Sporothrix cryptarchum* was isolated from wounds on hardwood trees and nitidulid beetles (*Coleoptera*: *Nitidulidae*), which visited fresh wounds on *Quercus robur*.

***Sporothrix undulata*** **R. Jankowiak & A. Ostafińska, sp. nov.**
MycoBank No: 840478
Fig. 10

**Etymology.** Referring to the aerial mycelium growing in undulating concentric zones on MEA.

**Type.** Poland, Małopolskie Province, Wierzchosławice, from *Epuraea guttata* on *Quercus robur*, June 2016, *R. Jankowiak*, (O-F-258635 ***holotype***, culture ex-type CBS 147929).

**Description.** Sexual and asexual structures produced on sterilised oak twigs and on surface of malt agar in Petri dishes. *Ascomata* abundant, superficially or partly embedded in the agar, single or in groups; *ascomatal base* black, globose, (65–)95–186(–223) µm diam., with brown hyphal hairs, 8 to 134 µm long and 1.2 to 3.1 µm wide at the base; *ascomatal necks* black, straight or curved, (114–)174–482(–697) µm long, diameter (9.1–)12.3–18.7(–24.2) µm at the apex and (14.7–)22–40.3(–58.7) µm at the base. *Ostiolar hyphae* present, pale brown, with small granules, septate, straight or slightly waved, tapering towards the apex or sporadically dichotomously branched at the tip, (9–)16–28(–31) in number, (29.4–)39.9–59.5(–72) µm long, (0.4–)0.6–1(–1.1) µm at the apex and (1.5–)1.8–2.7–(3.3) µm at the base. *Asci* subglobose to ovoid, (5.7–)6.7–8.5(–9.4) × (3.4–)4.4–5.8(–6.4) µm. *Ascospores* one-celled, kidney-shaped to almost triangular in side view (3.4–)3.8–4.6(–4.9) × (1.1–)1.4–1.7(–2) µm, elliptical in front view (3.2–)3.5–4.5(–5.6) × (0.9–)1.5–2.1(–2.8) µm, sometimes with residual sheath up to 0.6 µm thick, accumulated in white-colored mass at the tip of the neck. *Conidiophores* hyaline, micronematous or semimacronematous, simple or occasionally branched and bearing several conidiogenous cells, either borne on vegetative hyphae or on upright hyphae. *Conidiogenous cells* blastic, cylindrical, terminal, lateral or intercalary, straight or curved, slightly tapering towards the apex, swollen apical part forming conidia by sympodial proliferation on small or hardly visible denticles, (5.2–)11.3–50.4(–112.2) µm long, (0.9–)1.3–1.8(–2.1) µm wide at the base. Apical part (1.1–)1.6–3.4(–5.9) µm long and (1.1–)1.7–3.5(–5.4) µm wide. *Conidia* of two types: 1) sparsely in cultures, hyaline, unicellular, smooth, ellipsoid, pointed at the base, (3.2–)4.2–7.8(–11.7) × (1.4–)1.7–2.4(–3.5) µm, formed directly on denticles; 2) abundant in cultures, subhyaline to lightly pigmented, unicellular, smooth, subglobose to globose, sometimes pointed at the base, (2.1–)2.9–4.2(–5.5) µm diam, formed singly or in chains, either directly on the side of vegetative hyphae, on short lateral branches or denticles. *Culture characteristics*: Cultures showing optimum growth at 25 °C (1.2 mm/d) with growth somewhat slower at 20 °C and 30 °C (0.9 mm/d), white or white grey, flat, growing in a circular pattern with undulate margins.

**Host tree.** *Alnus glutinosa*, *Carpinus betulus*, *Fagus sylvatica*, *Quercus robur*, *Quercus rubra*, *Salix fragilis*.

**Insect vector.** *Cryptarcha undata*, *Epuraea guttata*.

**Distribution.** Poland.

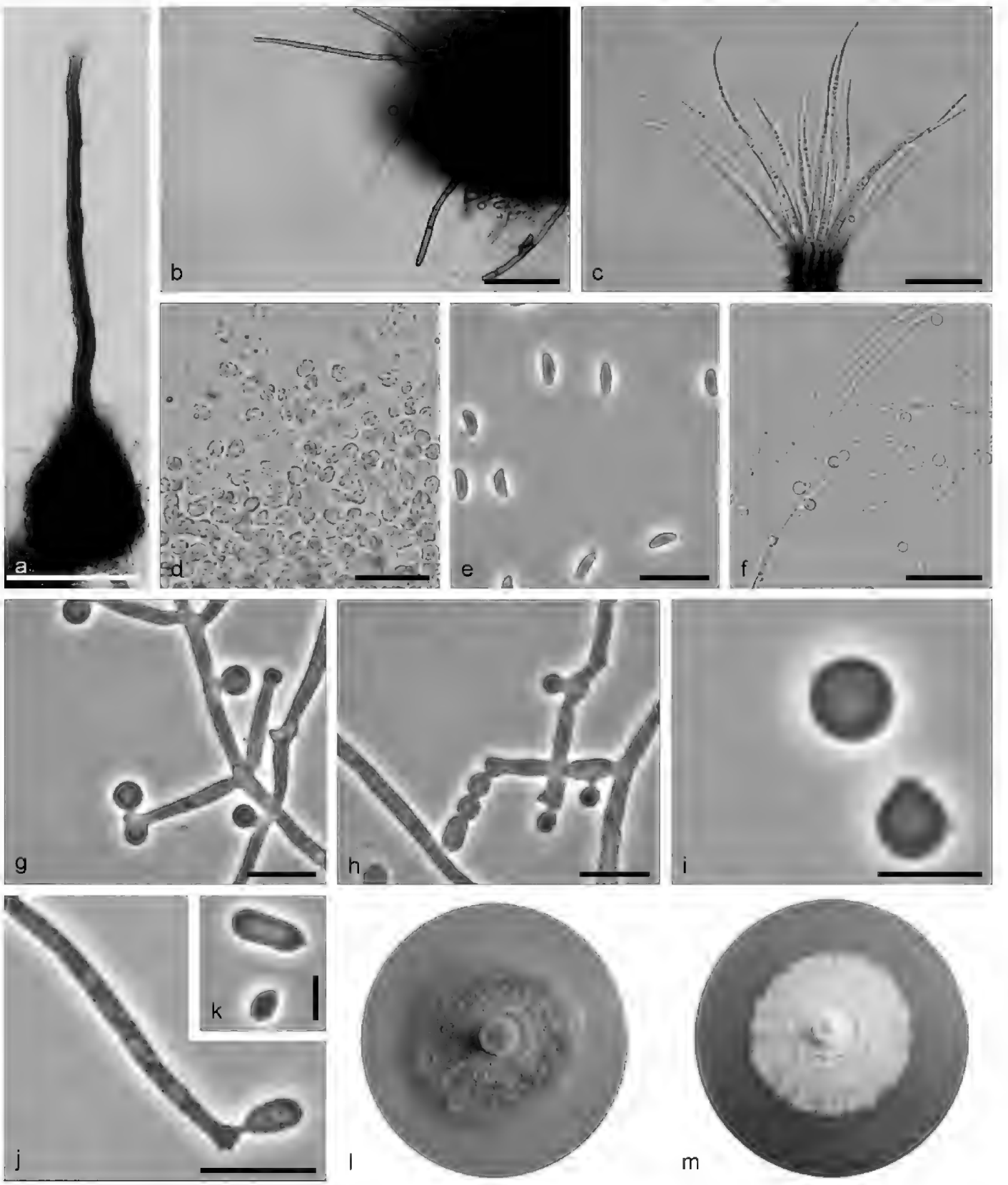


**Figure 10.** *Sporothrix undulata* sp. nov. (CBS 147929) **a** ascoma **b** ascomatal base **c** ostiolar hyphae **d** asci **e** ascospores **f–h** globose conidia arising on long conidiophore or directly from hyphae **i** globose conidia **j** conidiogenous cell with an inflated cluster of denticles at the apex **k** conidia **l–m** fourteen-day-old culture on MEA (left- pigmented CBS 147929, right – white KFL404DB16bRJCU). Scale bars: 100 µm (**a**), 25 µm (**b–d**), 10 µm (**e**), 25 µm (**f**), 10 µm (**g, h**), 5 µm (**i**), 10 µm (**j**), 5 µm (**k**).

**Additional specimen examined.** Poland, Małopolskie Province, Wierzchosławice, from wound on *Quercus robur*, October 2015, *R. Jankowiak* (O-F-258636, cultures CBS 147931).

**Notes.** This species is phylogenetically distinct from the other *Sporothrix* species based on the ITS, βT, CAL and TEF1-α sequences. *Sporothrix undulata* is

phylogenetically closely related to *S. cryptarchum* described in this study. The morphological differences between *S. undulata* and *S. cryptarchum* are described in the section above treating *S. cryptarchum*.

*Sporothrix undulata* was represented by nine isolates collected from Poland. It corresponds to *Sporothrix* sp. 12 in the study of Jankowiak et al. (2019b). In this study *S. undulata* was isolated from wounds on hardwood trees and from adults of nitidulid beetles (*Coleoptera*: *Nitidulidae*), which visited wounds on *Quercus robur*.

***Sporothrix cavum*** **R. Jankowiak sp. nov.**
Mycobank: 840479
Fig. 11

**Etymology.** From Latin, referring to the hollow cavities produced by woodpeckers and from which this fungus was collected.

**Type.** Poland, Małopolskie Province, Kraków, from the cavity of *Dendrocopos major* on *Salix fragilis*, December 2015, *R. Jankowiak*, (O-F-258637 ***holotype***, culture ex-type CBS 147943).

**Description.** Sexual morph not observed. Asexual structures produced on sterilized beech twigs placed on the surface of malt agar in Petri dishes. *Conidiophores* hyaline, micronematous, simple, straight, simple or branched, bearing several conidiogenous cells, either borne on vegetative hyphae or on upright hyphae. *Conidiogenous cells* blastic, cylindrical, terminal, lateral or intercalary, straight or curved, slightly tapering toward the apex, swollen apical part forming conidia by sympodial proliferation on well-developed denticles, (2.8–)11.5–32.8(–54.4) µm long, (0.7–)1.1–1.7(–2.4) µm wide at the base. Apical part with denticles (1.2–)1.5–2.8(–4.4) µm long and (1.4–)1.8–2.6(–3.1) µm wide, individual denticles often formed below aplical part. *Conidia* hyaline, unicellular, smooth, obovoid, with pointed bases, (3.1–)3.6–5.5(–7.8) × (1.7–)2–2.7(–3.2) µm, formed on terminal or lateral denticles, either directly on the side of vegetative hyphae. *Culture characteristics*: Cultures having optimum growth at 25 °C (1.7 mm/d) followed by at 30 °C (1.5 mm/d), growing well at 35 °C (0.6 mm/d), greyish green, with a darker centre, flat, growing in a circular pattern with smooth margins and abundant aerial mycelium.

**Host tree.** *Malus domestica*, *Salix fragilis*

**Insect vector.** unknown

**Distribution.** Poland

**Additional specimen examined.** Poland, Małopolskie Province, Książ Wielki, from the cavity of *Dendrocopos medius* on *Malus domestica*, (O-F-258638, cultures ex-paratype KFL=NRFI 35614DR).

**Notes.** This species is phylogenetically distinct from the other *Sporothrix* species based sequences for the ITS, βT, CAL and TEF1-α regions. *Sporothrix cavum* is related to *S. polyporicola* based on analyses of the ITS sequences. However, *S. cavum* in contrast to *S. polyporicola*, does not produce a sexual morph (Constantinescu and

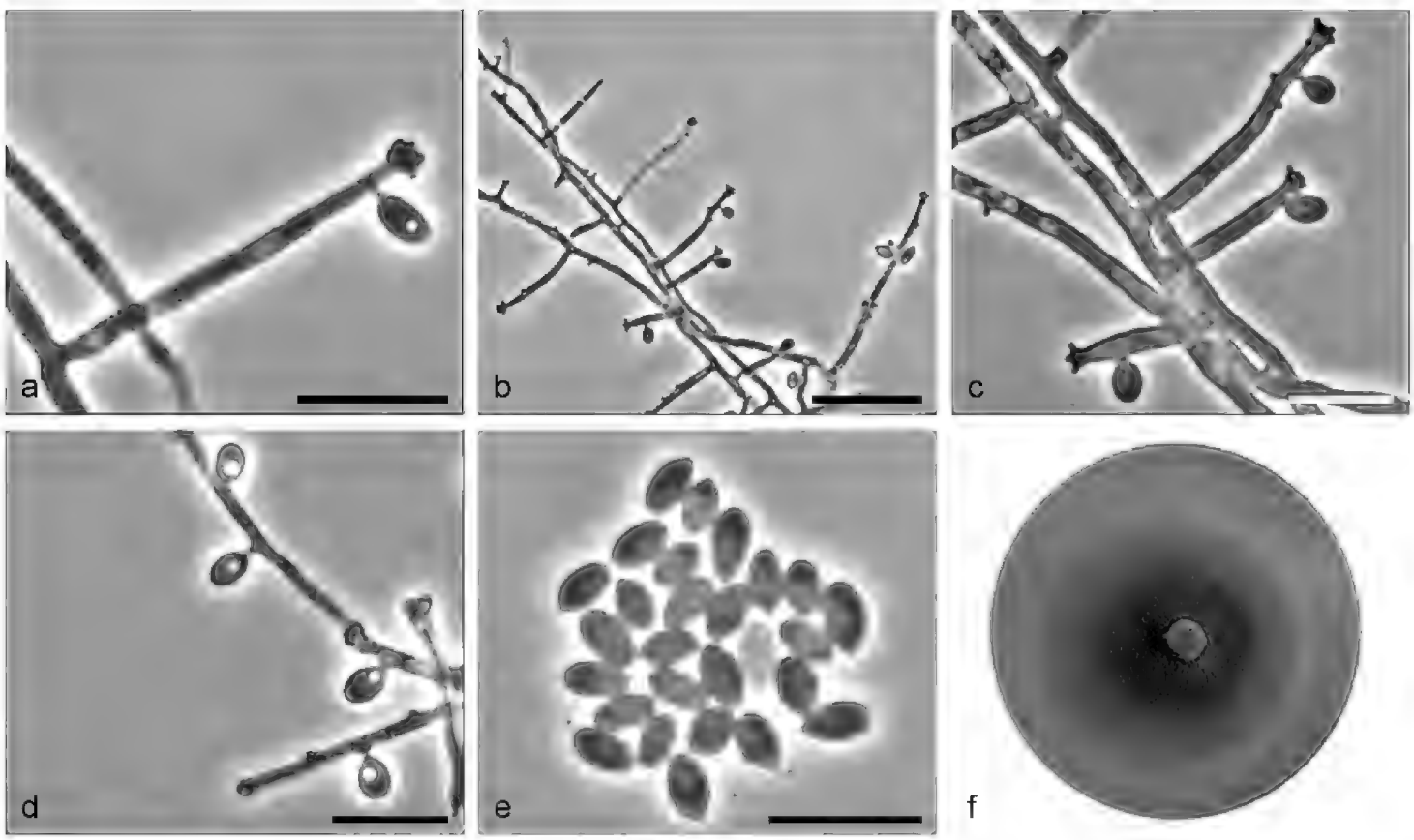


**Figure 11.** *Sporothrix cavum* sp. nov. (CBS 147943) **a–c** conidiogenous cell with an inflated cluster of denticles at the apex and below apex **d** conidiogenous cells arising directly from hyphae **e** conidia **f** fourteen-day-old culture on MEA. Scale bars: 10 μm (**a**), 25 μm (**b**), 10 μm (**c–e**).

Ryman 1989). In addition, *S. cavum* has obovoid and short conidia (3.1–7.8 μm), whereas *S. polyporicola* has clavate and longer conidia (6–14 μm) (Constantinescu and Ryman 1989).

*Sporothrix cavum* was represented by two isolates collected from the cavities produced by the woodpeckers *Dendrocopos major* on *Salix fragilis* and *Dendrocopos medius* on *Malus domestica*. It corresponds to *Sporothrix* sp. 18 in the study of Jankowiak et al. (2019c).

## Discussion

Our work (Jankowiak et al. 2019a, 2019b, 2019c; this study) has led to the discovery of six novel *Sporothrix* species associated with hardwood trees in Poland. Description of these new species brings the total number of species in this genus to 62, of which 16 occur in Poland. These include the six species described here as well as *S. aurorae* (Jankowiak et al. 2019b), *S. cantabriensis* (Jankowiak et al. 2017), *S. dentifunda* (Aghayeva et al. 2005, Jankowiak et al. 2019b), *S. eucastaneae* (Jankowiak et al. 2019a, 2019b, 2021), *S. fusiformis* (Jankowiak et al. 2019a, 2019b), *S. inflata* (Jankowiak et al. 2012; Jankowiak and Bilański 2013a, 2013b), *S. inflata* '2' (Jankowiak et al. 2019a, 2019b), *S. prolifera* (Kowalski and Butin 1989; Jankowiak et al. 2019a, 2019b), *S. stenoceras*, (Kowalski and Butin 1989; Jankowiak and Bilański 2013b, Jankowiak et al. 2019b) and *S. variecibatus* (Jankowiak and Bilański 2013b).

All of the species described in this study are morphologically similar, having asexual states with hyaline or lightly pigmented conidia produced holoblastically on denticulate conidiogenous cells or directly from the hyphae. Where ascomata were present, these tended to have globose bases with elongated necks terminating in long ostiolar hyphae and allantoid or kidney-shaped ascospores not surrounded by hyaline sheaths. All of the newly described species grew optimally at 25 °C and all also grew well at 30 °C on MEA. *Sporothrix undulata* and *S. cavum* differed from the other four species in having pigmented as opposed to white cultures on MEA. All of the newly described species were recovered from hardwood ecosystems in Poland in association with bark and ambrosia beetles, nitidulid beetles, naturally occurring tree wounds or woodpecker cavities.

The six species described in this study can easily be distinguished from each other and from the other species of *Sporothrix* based on the DNA sequence comparisons. Analyses of the ITS sequence data were insufficient to distinguish between *S. cryptarchum* and *S. undulata* or between *S. cracoviensis* and *S. fusiformis*. However, analyses of sequence data for the protein-coding genes, including the βT, CAL and TEF1-α showed that *S. cracoviensis*, *S. cryptarchum*, and *S. undulata* represent distinct taxa. Furthermore, the two closely related species, *S. cryptarchum* and *S. undulata* formed a new and well-supported lineage in *Sporothrix* including species infecting wounds on a variety of hardwood trees. The species in this lineage are characterised by having both hyaline as well as pigmented conidia and kidney-shaped ascospores.

The asexual morphs of the *Sporothrix* species described in this study had variable morphology. All species had hyaline conidia produced holoblastically on denticulate conidiogenous cells that proliferate sympodially or arise directly from hyphae. *Sporothrix cryptarchum* and *S. undulata* also had pigmented globose conidia formed singly or in chains, either directly on the sides of the vegetative hyphae or on short lateral branches. The presence of two different conidial types has previously been found in other *Sporothrix* species, including *Sporothrix dimorphospora* and *S. brunneoviolacea* (Madrid et al. 2010) as well as *S. brasiliensis*, *S. globose*, and *S. mexicana* (Marimon et al. 2007).

Recently, Jankowiak et al. (2019b) provided evidence that fresh wounds on hardwood trees in Europe are preferred habitats for some *Sporothrix* species. These authors isolated 15 *Sporothrix* species from trees belonging to 12 species of angiosperms. Likewise, nine *Sporothrix* species have been described from fresh wounds on non-native *Eucalyptus* spp. and various genera of native trees in South Africa (Kamgan Nkuekam et al. 2012; Musvuugwa et al. 2016, 2020; Osorio et al. 2016).

Three species of wound-associated *Sporothrix* spp. collected during a survey reported in the study of Jankowiak et al. (2019b) were included in the present study. The greatest number of isolates (194) obtained during that survey were those of *S. undulata*. This species was found as a common associate of bleeding wounds on *Quercus robur* and *Salix fragilis*, suggesting that they might have some level of pathogenicity. The other species inhabiting wounds on hardwood trees that was collected during the survey of Jankowiak et al. (2019a) was *S. cryptarchum* (34 isolates). Transfer of this species to the sampled tree wounds was most likely by nitidulid (*Coleoptera*, *Nitidulidae*) beetles as previously noted by Jankowiak et al. (2019b) who suggested

that these insects commonly transmit *Ophiostomatales*, including *Sporothrix* species to tree wounds in Poland. Likewise, Kamgan Nkuekam et al. (2012) have demonstrated that the nitidulid beetles *Brachypeplus depressus* and *Carpophilus* spp. vector *S. candida* and *S. fumea* in the *Eucalyptus* plantations of South Africa. This association is also consistent with other studies providing compelling evidence that nitidulid beetles act as vectors of the well-known pathogens, such as *Bretziella fagacearum* (De Beer et al. 2017; Jagemann et al. 2018) and *Ceratocystis albifundus* (Heath et al. 2009).

The second largest number of isolates (81 in total) included in this study represented two species in the *S. gossypina*-complex, bringing the total number of species in that complex to 15 (De Beer et al. 2016; Wang et al. 2019). *Sporothrix cracoviensis* was represented by 45 isolates from the ambrosia beetles *Trypodendron domesticum* and *T. signatum* collected on *Fagus sylvatica* (Jankowiak et al. 2019a). This is not unusual given that an association between ambrosia beetles has recently been recorded by De Errasti et al.(2016) in a study on *Nothofagus pumelo* in Patagonia. The other species residing in this complex collected during the survey of Jankowiak et al. (2019a) is *S. fraxini* (36 isolates). This fungus was found on *Fraxinus excelsior* in association with the bark beetles *Hylesinus crenatus* and *H. varius* (Jankowiak et. al. 2019a).

The Polish study by Jankowiak et al. (2019a) revealed that, apart from *S. cracoviensis* and *S. fraxini*, five other *Sporothrix* species (*S. fusiformis*, *S. prolifera*, *S. eucastanea*, *Sporothrix* sp. 4, *Sporothrix* sp. 9) were associated with bark beetles. These findings confirm that most species in the *S. gossypina* complex are associated with galleries of conifer-infesting bark beetles worldwide (De Beer et al. 2016). The other species in the *S. gossypina*-complex were isolated from the stained oak wood (Kowalski and Butin 1989; Aghayeva et al. 2004), cankers caused by *Cryphonectria parasitica* on chestnut (Davidson 1978), a hardwood tree native to South Africa (Musvuugwa et al. 2016), and from mites infesting the infructescences (flower heads) of *Protea* in South Africa (Roets et al. 2008).

*Sporothrix cavum*, the remaining taxon collected from hardwood trees during the surveys that formed the basis of the present study, resided in lineage F defined by De Beer et al. (2016). This lineage includes three species, namely *S. polyporicola*, *S. dimorphospora*, and *S. inflata* '2'. Two of these species (*S. dimorphospora*, and *S. inflata* '2') are known from soil and *S. polyporicola* was isolated from basidiocarps of the polypores *Fomitopsis pinicola* and *Amaropostia stiptica* (Constantinescu and Ryman 1989; Madrid et al. 2010). The results of the present study show that species in this complex also accommodate wood-inhabiting *Sporothrix* species. Other than the fact that *S. cavum* was isolated from cavities on *Salix fragilis* and *Malus domestica* made by woodpeckers (Jankowiak et al. 2019c), nothing is known regarding the ecology or distribution of the fungus. It could, for example, be introduced into these cavities by arthropods or have some relationship with the woodpeckers themselves.

The results of this study have substantially expanded our knowledge of *Sporothrix* and the ecology of species in this genus. Broadly, the results suggest that *Sporothrix* species are common members of the *Ophiostomatales* in hardwood ecosystems in Poland. Furthermore, interesting questions have arisen that should shape future investigations regarding these fungi.

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